

THE TIME-TEMPERATURE RELATION OF DIFFERENT STAGES OF DEVELOPMENT

FRANCIS JOSEPH RYAN

(From the Department of Zoölogy, Columbia University, New York City)

The phenomenon of development appears as a series of processes which are visibly unlike. Many investigators have demonstrated that some of these events can also be separated on the grounds that their rates possess different temperature coefficients. For example, the rate of growth of the gill filaments in the frog is more depressed by a low temperature than is the rate of body growth (Atlas, 1935; Doms, 1915). Again, the rate of embryo formation in *Salmo* has a higher temperature coefficient than the rate of growth in wet weight (Gray, 1928). In view of this and other evidence it has always seemed curious that development could yield approximately the same differentiated product over a wide range of temperatures. As a possible solution to the problem, Tyler (1936a) has shown that in some marine invertebrates the temperature coefficients of various cleavages are not only the same but are also identical with those for later stages of differentiation. Yet his results are not comparable with those on the frog obtained by Hertwig (1898) and Krogh (1914) whose data show, although the authors do not point it out, that the temperature relation of cleavage is different from that of later development. The experiments on the egg of the frog to be reported here were designed to discover the temperature relations of some of the more clear-cut events of development which could be accurately measured.

Rana pipiens from Vermont were caused to ovulate at 15° by pituitary injection. Batches of about 25 eggs were shed into finger bowls and fertilized artificially. The sperm suspension was replaced after 5 minutes by 200 cc. of 10 per cent Ringer's solution at the temperature at which the eggs were to be kept and the bowls were then distributed to constant temperature environments. When the jelly swelled, the egg mass in each container was cut into bunches containing about 5 eggs apiece. The 10 per cent Ringer's was replaced daily by solution at the same temperature. Cold rooms, water baths and incubators were used to maintain constant temperatures. Generally the temperatures were constant within 0.1° C. (except at 10° and 8.5° where ice-boxes were used; the maximum observed variations here were 1.0° and 0.5° re-

spectively). The temperature in the finger bowls seldom changed more than 0.5° during observation on the stage of a binocular microscope and observation never lasted as much as 5 minutes. Mortality during early development was less than 5 per cent and development was normal at temperatures between 25° and 8° . Above and below these temperatures mortality increased and abnormalities became frequent, so that at 29.6° usually less than 50 per cent of the eggs hatched and abnormalities were very common.

The times to various cleavages, gastrulation and gill circulation were measured from fertilization for embryos remaining constantly at one of the several temperatures. Cleavage was considered begun when the first slight furrowing was seen on the surface of the egg; gastrulation when the dark line of pigment associated with the initial dorsal lip invagination appeared; and gill circulation when the initial blood corpuscles could be seen circulating in the anterior gill. When the critical time approached, repeated observations were made until about 50 per cent of a batch of eggs had reached the initiation point, at which time it was considered that the stage was entered. All of the embryos in a group of 25 entered a stage well within 10 per cent of the total time necessary to reach that stage. The maximum deviation of any batch of eggs from the average time was about 10 per cent.

In order to portray the relation between the times to different stages at different temperatures a semi-logarithmic plot was chosen (see figures). The logarithm of time was placed along the ordinates and the abscissae represent either temperature, in which case the curves are for different developmental intervals, or stages, in which case the curves are for different temperatures. The choice of one of these abscissae was made so as to employ the largest number of points per curve. In either event it is possible to compare what types of function of time the stages are at different temperatures. Spacing of temperatures along the abscissa was obtained by plotting the data for one stage as a straight line. This arbitrary abscissa was then used as a base for the times to other stages. Stages were spaced along the abscissa in a similar fashion. This method is preferred to the comparison of temperature coefficients inasmuch as: (1) it does not entail a selection of points but involves all of the data; (2) it avoids attributing one of the several controversial numerical constants to the temperature relation; and (3) the linear arrangement of points obtained by a distortion of one axis permits immediate visual comparison of the time-temperature relation.

Figure 1 compares the temperature relation of the different stages of development in *Rana pipiens*. The curve for time between gastrulation and gill circulation (Stages 10-20) has the greatest slope. The curve

for time between fourth cleavage and gastrulation has a lesser slope which is, however, greater than the slope of the curves for all the cleavages. These differences are real. If the curves actually were parallel to that for gastrulation to gill circulation, a time error of 25 per cent would have to be assumed at both ends of the gastrulation curve, an error of about 45 per cent at both ends of the curve for first cleavage and an error of about 45 per cent at both ends of the curve for second, third and fourth cleavage. Such errors are highly improbable because the

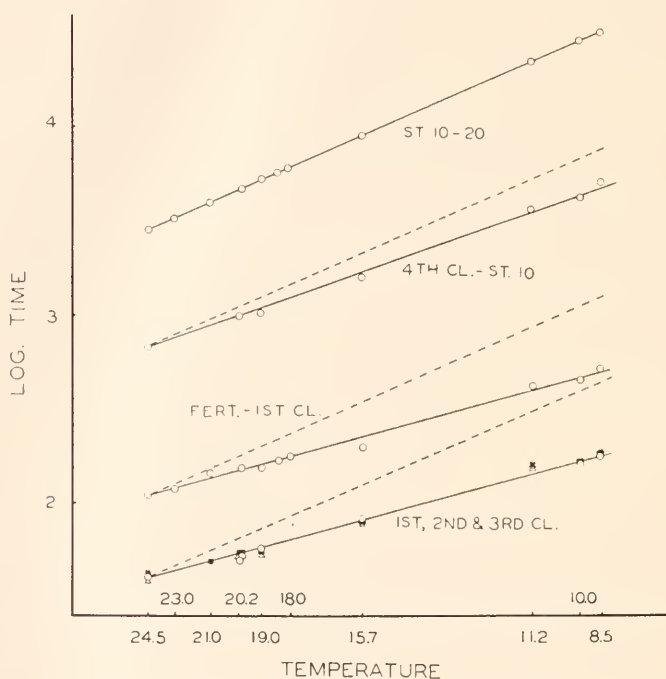


FIG. 1. The relation, at different temperatures, of developmental intervals to time in *Rana pipiens*. Ordinate, logarithm of time in minutes between the specified stages; abscissa, temperature in °C. The data for development between stages 10 and 20 are plotted as a straight line by the arbitrary distortion of the temperature axis. The latter is used as a base for the data for other intervals. The upper complete curve describes development between gastrulation (stage 10 of Pollister and Moore, 1937) and gill circulation (stage 20); the next, between fourth cleavage and gastrulation; the next, between fertilization and first cleavage. In the lowest curve, the circles describe development between first and second cleavage, the squares, between second and third, and the triangles, between third and fourth. Twenty points are single determinations; the remaining thirty-four points are the averages of from two to ten determinations. One hundred and seventy-two determinations were made in all. The broken lines were drawn through the points at 24.5° parallel to the curve for stages 10 to 20. These broken lines emphasize the real nature of the slope differences among the curves for different intervals.

maximum deviation of a point from any one of the curves is only 10 per cent. The deviations of points for the cleavages from the parallel curves drawn through them are at random and are about the size of the expected experimental error (10 per cent). Hence, in *Rana pipiens* the temperature relations of cleavages are alike, but they are different from those of later development.¹ Differences in temperature relation are apparent even in rate-temperature plots (Ryan, 1941) where, in addition to a difference in μ values, the curve for later development shows a "break" at about 18° C., while the curves for cleavage "break" around 14° C.

The data of Krogh (1914) for *Rana butyrhina* when placed on the semi-logarithmic plot (Fig. 2) completely confirm this difference between cleavage and later development. Even though the times to later stages are from fertilization and must include some time during cleavage when the temperature relation is like that of the upper curve, a significant difference in slope is visible. If all the curves actually were parallel, an error of about 25 per cent must be postulated at both ends of the cleavage curve. Such errors are extremely improbable with Krogh's method. His precision in measuring cleavages should be better than that for later stages and yet no such errors are visible in his later stage data (for example, the maximum deviation of a point from the straight lines in Fig. 2 is equivalent to an error in time of only 5 per cent). Despite this difference between cleavage and later development, the parallelism of curves among different stages of later development in Fig. 2 shows that the temperature relations of the latter are alike.² Apparently in contradiction to this, Bělehrádek (1926) has calculated for Krogh's data a series of b values increasing from 1.76 to 2.52 between medullary groove closure and 7.8 mm. tadpole formation. However, from the same data and for the same stages b values of 1.6 and 1.7 (and Q_{10} 's decreasing from 4.2 to 3.5) can be calculated according to the points selected for comparison.

¹ Atlas' (1935) Fig. 8 indicates the same temperature characteristic over the low temperature range for the rates of different stages of development in *Rana pipiens*, but the column he uses to include all the points obscures the difference between the temperature relations of the different stages. At higher temperatures the temperature relations of cleavages, of gastrulation, and of later development show the same sort of differences as are visible in Fig. 1 of this paper. Neither the data for *Rana pipiens* described in this paper nor Atlas' data (Fig. 2) for the same animal show the adaptation in the rate of later cleavages found by Hoadley and Brill (1937) in *Arbacia* and *Chactopterns*, although this may be because the temperatures used were not close enough to the maximum.

² Times from fertilization or first cleavage, instead of the length of developmental intervals, are used in this and all subsequent figures in order not to exaggerate errors in timing the events of later development which are difficult to measure.



Confirmation of the similarity in the temperature relations of events in later development can be found in semi-logarithmic plots of the data of Moore (1939) for *Rana pipiens*, *R. sylvatica*, *R. clamitans* and *R. palustris*, and of Knight (1938) for *Triton alpestris*. The curves for

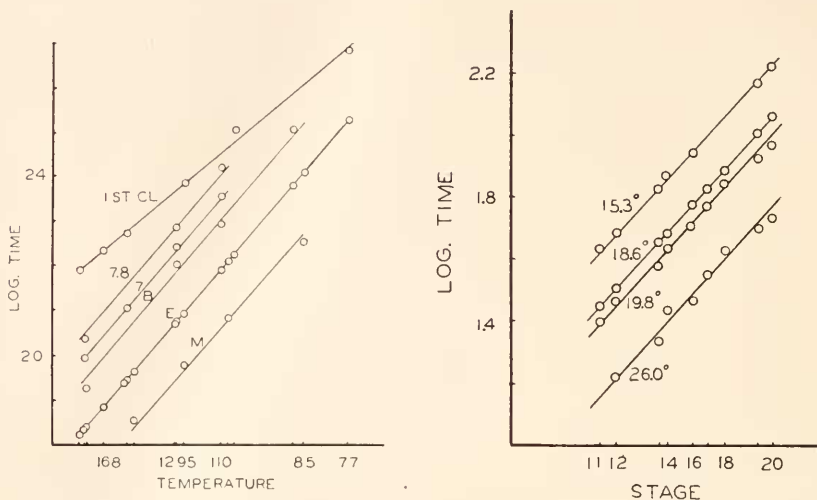


FIG. 2 (left). Krogh's (1914) data for *Rana butyrhina* showing the relation at different temperatures of stage to time from fertilization.³ Ordinate represents the logarithm of time from fertilization (for cleavages in minutes, for later stages in hours); abscissa, temperature in °C. The symbols over the curves represent first cleavage, 7.8 and 7 mm. length, branched gills, external gills, and medullary groove closure. For the sake of ready comparison, the data for the formation of external gills are plotted as a straight line by the arbitrary distortion of the temperature axis. The latter are used as a base for the data for other stages.

FIG. 3 (right). Moore's (1939) data for *Rana pipiens* showing the relation, at different temperatures, of stages between gastrulation and gill circulation to time from first cleavage. Ordinate represents time from first cleavage in hours; abscissa, stage of development (Pollister and Moore, 1937). For the sake of ready comparison the data for 18.6° are plotted as a straight line by the arbitrary distortion of the stage axis. The latter are used as a base for the data from other temperatures.

later development are all parallel. For example, in Fig. 3 Moore's data for *Rana pipiens* are presented. These supplement the data in

³ It should be pointed out that the similarity in temperature relation among these stages does not necessarily imply that each step in the formation of a given stage has the same temperature relation. There may be differences of short duration which might be in opposite directions and cancel one another. At any rate, if there are such differences, they are not additive, for the overall sort of examination made does not reveal them. The stages in between gastrulation and stage 20 are not clear-cut enough to obtain easily sufficiently accurate determinations to solve this problem.

Fig. 1 inasmuch as they show that many different events between yolk plug and gill circulation have the same temperature relation in *Rana pipiens*. Indeed, the significance of the difference between the temperature relation of the period from fourth cleavage to gastrulation and that of the interval between gastrulation and gill circulation (Fig. 1) is dubious. The process of gastrulation might have the same temperature relation as other stages of later development, but when the measured time also includes cleavages (between fourth cleavage and gastrulation), the observed overall temperature relation would be intermediate. Assuming that the curve for fourth cleavage to gastrulation is just such a composite, a calculation shows that the cleavage temperature relation would prevail into early blastula stages. However, it is very difficult to measure blastula stages accurately enough to break this period into its components and settle the problem.

Peter (1905) computed the Q_{10} 's for Hertwig's (1898) data on *Rana fusca* and claimed that the temperature coefficients gradually increased with the age of the animal. However, when the Q_{10} 's between 15° and 24° are compared for different cleavages, there is no significant difference from the average of 1.37. The same holds for later stages where there is no significant difference from the average of 2.36. Peter's difficulty resides in the fact that he included Q_{10} 's computed for low lethal temperatures where development began but was never completed. In this range the difference between high and low temperatures becomes progressively greater with age. When Hertwig's data are put on a semi-logarithmic plot (Fig. 4), it can be seen from the parallelism of curves that the times to stages of later development are the same type of function of temperature but are a different type from that for times to the first three cleavages. Hertwig could have a 25 per cent time error at both ends of each of his three cleavage curves. But since the maximum deviation of a point from the straight lines in Fig. 4A is only 10 per cent, the difference between cleavage and later development is probably real. The displacement of the points for gastrulation from the curves in Fig. 4B may be real (at 10° there is a time discrepancy of 20 per cent). This is in accordance with the fact shown in Fig. 1 that the rate of gastrulation increases to a degree intermediate between cleavage and later development with a temperature rise. Since gastrulation is compared with cleavage in Fig. 4A, the difference between the temperature relations of cleavage and later development is all the more convincing. In summary, it is definite that in Amphibia not only is the temperature relation different for cleavages and later stages, but there are extremely long periods during cleavage and during embryo formation over which the temperature relation remains constant.

It is not surprising that a difference should exist between the temperature relations of cleavages and morphogenesis because visibly these phenomena are unlike and probably have different causes. The amazing thing is the similarity in response of so many different stages to temperature. Second, third, and fourth cleavages are enough alike so that it is not hard to believe that they are the result of the same process. Between fertilization and first cleavage, however, there occur the completion of the second maturation division, release of the second polar

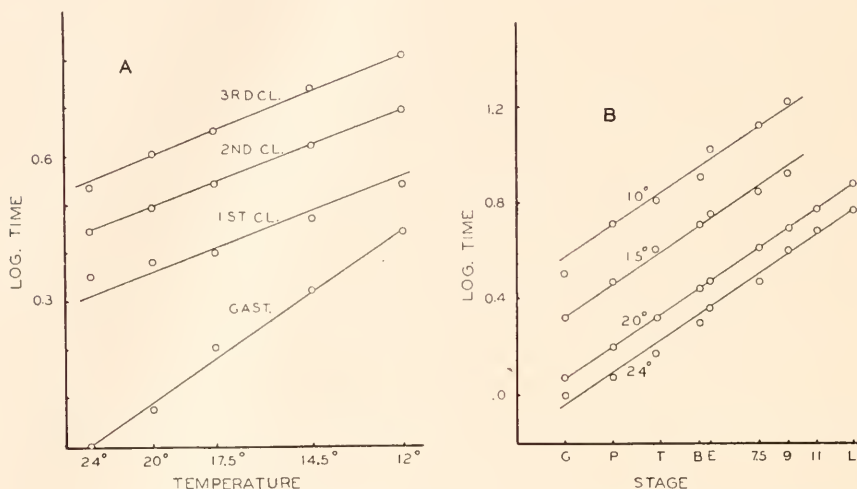


FIG. 4. Hertwig's (1898) data for *Rana fusca* showing the relation, at different temperatures, of stage to time from fertilization. A. Ordinate represents logarithm of time from fertilization (for cleavages in hours, for later stages in days); abscissa, temperature in °C. B. Ordinate, logarithm of time from fertilization in days; abscissa, stage of development from gastrulation to limb bud formation. At temperatures of 6° and below the parallel relation does not hold, but also development does not go to completion at these temperatures. The abscissae in A and B, using the data for second cleavage and for 20° respectively as the base curves, have been distorted as in Figs. 1 and 3.

body, and the fusion of pronuclei, before the process begins to resemble later cleavages. Since the total process of first cleavage is, then, different from that of later cleavages, it would be expected *a priori* that the temperature relations should differ. But they do not (Fig. 1). The coincidence may be chance or due to the independence of the cytoplasmic cleavage (which is being measured) from the nuclear phenomena (wherein lies the difference between first and later cleavages) or due to a fundamental process which controls all of the phenomena and imposes its temperature relation upon them. The latter seems more likely

inasmuch as it affords an explanation of the even more astonishing similarity in the temperature relations of stages between gastrulation and stage 20 (Fig. 3). Here such strikingly different processes as neurulation, tail bud and gill formation, onset of circulation etc. have the same relation. Either each operation independently has achieved this or all are controlled by an underlying process which imposes its temperature relation upon its various expressions. Atlas (1938) has shown that in *Rana pipiens* the temperature coefficient of the rate of oxygen consumption during development is approximately the same as that of the rate of development. In many marine eggs temperature affects the rate of early development and the rate of respiration in the same way (Tyler, 1936b). These correlations suggest that the "primary gear shaft" (Needham, 1933) integrating developmental processes is some part of the respiratory metabolism.

If this were so, then there should be a difference in the type of metabolism during cleavage in the frog's egg from that prevalent during morphogenesis. Brachet (1934) has, indeed, shown that in the frog the respiratory quotient changes abruptly at gastrulation from about 0.7 to 1.0. Again, in Tyler's (1936a) studies of marine invertebrates, there should be the same type of metabolism during cleavage as during later development because the temperature relations of both processes are the same. Accordingly, in *Urechis* where the temperature coefficients of the first four cleavages are the same (Tyler), the respiratory quotient remains unchanged over an equivalent period of time (2½ hours at 20° C.) (Horowitz, 1940). Thus there is real evidence for the belief that the coördinator of the various processes of differentiation, the factor which permits development to be reproducible over a wide range of temperatures, is the respiratory metabolism.

SUMMARY

1. Stages in embryo formation among Amphibia between yolk plug and gill circulation have similar time-temperature relations.
2. The time-temperature relation of cleavage, although constant from first to fourth cleavages, differs from that of embryo formation.
3. It is suggested that the different time-temperature relations of cleavage and of morphogenesis represent different controlling processes; while the similarity of the time-temperature relations among cleavages and among stages of later development is the expression of a common controlling process in each case. These controlling processes are probably parts of the respiratory metabolism and they prevent temperature from disorganizing development.

I wish to thank Dr. H. B. Steinbach for criticisms of the manuscript.

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